

Molecular spring: from spider silk to silkworm silk

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In this letter, we adopt a new approach combining theoretical modeling with silk stretching measurements to explore the mystery of the structures between silkworm and spider silks, leading to the differences in mechanical response against stretching. Hereby the typical stress-strain profiles are reproduced by implementing the newly discovered and verified “ β -sheet splitting” mechanism, which primarily varies the secondary structure of protein macromolecules; Our modeling and simulation results show good accordance with the experimental measurements. Hence, it can be concluded that the post-yielding mechanical behaviors of both kinds of silks are resulted from the splitting of crystallines while the high extensibility of spider dragline is attributed to the tiny β -sheets solely existed in spider silk fibrils. This research reveals for the first time the structural factors leading to the significant difference between spider and silkworm silks in mechanical response to the stretching force. Additionally, the combination of theoretical modeling with experiments opens up a completely new approach in resolving conformation of various biomacromolecules.

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Natural silk spinning was pioneer of economy flourishing worldwide since the first industrial revolution, and was continuing acting as a pillar industry during the last several centuries until massive manufacturing of man-made fibres derived from feedstocks of petrochemical was popularized. Spider silk, superior to other biomaterials, has extraordinary strength comparable to steel, and the highest toughness among all natural silk fibres up to date[1, 2, 3, 4]. It is suggested that one strand of pencil thick spider silk can stop a Boeing 747 in flight. The exceptional properties serve new application in industry to fulfill various functions, such as bullet-proof vests, reinforced composites and aircrafts panels substitute. On the other hand, the high production of silkworm silk makes its irreplaceable stay in textile and other markets, despite its inferior properties to spider silk[5, 6]. Complementarily, the sustainable application has been penetrated in daily life, ranging from costume manufacture to clinical treatment. Both two kinds of silks exhibit greater environmental friendliness and bio-compatibility than man-made petrochemical materials, which implies the convenience of its synthesis, fabrication and recycle[7, 8]. Although spider and silkworm silk share a high degree of similarity in their chemical composition and microscopic structures, the two kinds of silks behave differently in their mechanical responses[9, 10, 11, 12]. To fulfill their potential functionalities requires a full understanding of the underlying mechanism; however, to date very few works have been engaged in this exploration.

Both spider silk and silkworm silk are primarily comprised of Alanine- and Glycine-rich polypeptides in the fibrils; Besides, natural spun silkworm silk contains two strands coated with sericin, and the spider dragline silk contains major ampullate(MA) and minor ampul-

late(MI) silk. X-ray diffraction and AFM probing[13] provide direct evidence on the molecular level that subtle deviation in amino acids assembly sequence may lead to significant difference in mechanical properties[14, 15, 16]. However, it is still not sufficient to fully explain the mechanical difference. As can be seen, the stress-strain profile of silkworm silk is segmented in two regions, separated by yielding point. In low stress regime, the silk fibre behaves linearly elastic[17], and skips into the post-yield region with glass state which is nonlinear and irrecoverable[18]. Different from silkworm silk, spider draglines show the work-hardening phenomenon in the post-yield region, describing its dramatic increase of the elasticity of spider silk when subjected to certain extent of stretch, after which the fibres turn softer again.

Accordingly, the mechanical differences of spider draglines and silkworm silk are also related to the high-order structures of protein macromolecules[5, 6, 15, 19, 20]. The key problem is to determine structural factors, which affect mechanical response to the external stress and cause the differences of the two kinds of silks. The secondary structure of silks can be divided into crystalline, primarily the β -crystallites, and non-crystalline (amorphous) domains (Fig.1(a), (b)), which contains random coils, α -helices and β -sheets[21, 22]. It is believed that the secondary structures are similar in both two kinds of silk fibres except that ordered β -sheets were solely observed only in the amorphous region of spider draglines[13, 20, 23]; certainly, the crystallinity, denoting fraction of crystalline region, and the sizes of β -crystallites are different. We notice that one β -sheet in the amorphous region of spider draglines are stacked by part of a single protein macromolecule, while the β -crystallites in the crystalline regions are formed by sev-

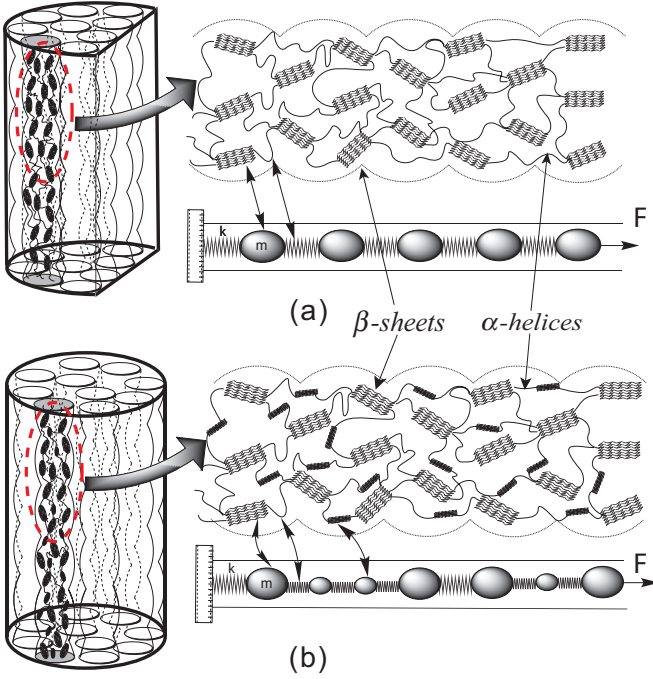


FIG. 1: The left panels are the structures of fibrils aligning along silkworm silk and spider silk fibres in (a) and (b) respectively. The upper-right sketches are the nano-structures of each fibril; the larger blocks denote the inter-protein β -crystallites, and the smaller beads denote the intra-protein β -sheets; the flexual lines in-between denote helical or random structures in the amorphous matrix. The lower-right parts is the model construction based on their nano-structures, and the element correspondence is directed by the thin solid arrows.

eral adjacent of them. In order to distinguish the two kinds of β -sheet structures, the one in crystalline region is referred to the “*inter-protein β -crystallite*”, and the one in amorphous region is referred to the “*intra-protein β -sheet*”, as shown in Fig.1(a) and (b).

In this letter, aiming at revealing the connection between the mechanical properties of spider and silkworm silk and their nano structures, we are inclined to understand the roles of the elements based on our models. Therefore, we are observing the collective behaviors of two kinds of silk, in terms of the structural difference, typically for the stress-strain profile.

Let us first start our modeling of silkworm silk. As shown in Fig.1(a), β -crystallites are extracted as massive bodies, and the in-between springs represent the amorphous matrix, including random coils, α -helices, etc. A quasi-periodic structure(Figure 1a) is adopted with repeated segments of rigid bodies and springs, and they are aligned along fibre axis subjected to the one dimensional confinement we simplified to. As indicated by Raman spectrum experiments, the β -crystallites and amor-

phous regions are more likely to connect in series[11], which is well described by the quasi-periodic structure. In general, the transition from rubber to glass state at the yielding point requires well definition in both the two regions. Hence, Hooke’s springs are imposed to describe the linear elastic behavior contributed mostly by amorphous region before reaching the yielding point, and become elasto-plastic with nonlinearity and irreversibility in the post-yield region. In addition, β -crystallites, previously treated as rigid bodies, are now actually elastic with relatively 4 times high elasticity of the whole fibre[10]. Taking into account the facts of the transverse size of β -crystallites shrinking and the irreversible process of elasticity measurement for the pre-stretched silk fibres, “ β -sheet splitting” turns to be possible mechanism in characterizing the yielding behavior[10, 11], and will be discussed in the following context.

Based on aforementioned facts, the model can be explicitly described as a serially connected system of N segments, each of which is comprised of massive body with mass m and light spring with original elasticity k_0 . The segment at one end is stretched by an applied force F and the other boundary one is fixed immobile. During the stretch process, for an arbitrary segment i , the extension linearly increases with the responsive force $F(i)$ in the linear region until $F(i)$ reaches a criteria of threshold force $F_{th}(i)$, after which the β -crystallites start to split. The collective behavior of splitting at $\{F_{th}(i)\}$ characterizes the critical behavior around the yielding point. Because the splitting of β -crystallites requires external energy input equal to or greater than the cohesive energy of hydrogen bonds, equivalently a large applied force F is likely to break more hydrogen bonds, thus to retrieve longer amorphous protein molecules from the compactly stacked β -crystallites. Hereby, a simple linear relation characterizing such observation between applied force F and the released molecule length $\Delta L(i)$ can be presumed as:

$$\Delta L(i) = \begin{cases} 0 & F < F_{th}(i) \\ (F - F_{th}(i))/E_0 & F \geq F_{th}(i) \end{cases} \quad (1)$$

Therefore, the relation between applied force F and observable extension Δx ($= \sum_{i=1}^N \Delta x_i$) can be deduced in Eq.(2) as:

$$\begin{aligned} F &= k_{eff} \cdot (\Delta x - \Delta L) \\ &= \frac{1}{\sum_{i=1}^N \frac{1}{k_i}} \cdot (\Delta x - \sum_{i=1}^N \Delta L(i)) \\ &= \frac{k_0 E_0 L_0 \Delta x - N k_0 L_0 \cdot \int_0^F (F - f) \rho_{th}(f) df}{N E_0 L_0 + N \int_0^F (F - f) \rho_{th}(f) df}, \end{aligned} \quad (2)$$

where $\rho_{th}(\cdot)$ is the distribution of the threshold force $\{F_{th}(i)\}$, L_0 is the original length of each segment, and

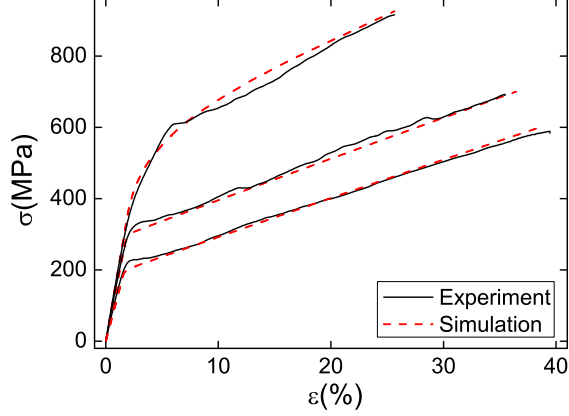


FIG. 2: The relation between stress σ and strain ϵ of silkworm silk fibres: the black solid line is the experimental curve, and the red dashed line is the simulation results. A computer-controlled motorized spindle was used to draw fibers from silkworm with 12mm/sec, 24mm/sec and 36mm/sec from the bottom up. Two successive 45min, 95°C heating procedures in 0.5% Na_2CO_3 and 1% soap solution was adopted in the degumming process. Measurements were performed using an Instron MicroTester (Model 5848; force resolution, 0.5% of indicated load; position resolution 0.02 μ m; strain rate is 50%/min), at $20 \pm 2^\circ C$ and the humidity was kept at $60 \pm 5\%$. the scaling constant in simulation $S_c = 20$, the corresponding parameters k_0 , E_0 , and $\langle F_{th} \rangle$ increase with the reeling speed.

$h(x)(\int_{-\infty}^x \delta(x)dx)$ is the step function. Besides, the calculation of elasticity k_{eff} follows the law of sequentially linked springs with effective elasticity of each segment $k_i = k_0/(1 + \Delta L(i)/L_0)$. Additionally, for large system size N , continuity approximation is applied in deriving Eq.2. Mapping three dimensional reality to our one dimensional model, the scaling is governed by such regulations: $F \sim \sigma$ (set the area of cross section $\Delta S = 1$), and $\Delta x \sim \epsilon \cdot NL_0$, where σ and ϵ denote the tensile stress and tensile strain respectively. As one of the key features of biomaterial, ununiformity caused by random generated defects leads to serious instability of their mechanical properties. Without loss of generality, gaussian random function is chosen for the distribution of threshold forces.

To mimic the dynamic process of silk stretching, the extensive numerical simulations based on Molecular Dynamics (MD) were carried out. In comparison, the stress-strain profiles of silkworm silk (Fig.2) and spider draglines (Fig.4) under different reeling speeds were measured. As shown in Fig.2, MD numerical results of stress-strain profiles coincide very well with experimental data at the proper values E_0/k_0 and $\langle F_{th} \rangle$. It is found that the ratio E_0/k_0 determines the relative steepness of the two-segmented stress-strain profiles, with two limits that the

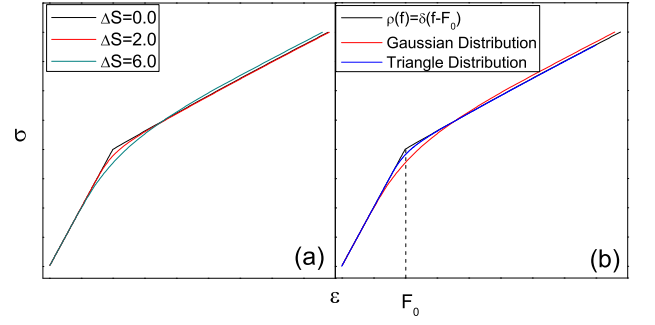


FIG. 3: (a): Stress-strain profile of silkworm silk for different spread ΔS of the gaussian form $\rho_{th}(\cdot)$ and same expectation value $\langle F_{th} \rangle$. (b): Stress-strain profile of silkworm silk for different forms of $\rho_{th}(\cdot)$ with same $\langle F_{th} \rangle$ and various spread.

post-yield behavior is strain-independent for $E_0/k_0 \sim 0$, and remains linear elastic for $E_0/k_0 \sim \infty$. The stress-strain profile is relatively robust to the form of distribution function of threshold forces, as general gradually varying and centralized functions give out similar profiles. As shown in Fig.3, various distribution of threshold force ρ_{th} gives no essential difference, but only little variation. Additionally, different reeling speed corresponds to their specific $\langle F_{th} \rangle$ and E_0/k_0 , reflecting the initial formation difference of the nano structures of macromolecules, presumably related to the β -crystallites position and orientation. Therefore, the breaking of β -crystallites in silkworm silk fibrils weakens the linkage between protein molecules, which leads to its softening in the post-yield regime.

To model the structure of spider dragline, we take into account the performance of the intra-protein β -sheets in the amorphous region (Fig.1(b)), which is uniquely observed spider draglines, in addition to adopting the idea of the above model. Analogous to the silkworm silk modeling, the linear assumption of protein molecule length split from intra-protein β -sheets $\{\Delta L'(i)\}$ versus stretching force F is preserved, with proportional constant E_1 and threshold forces of splitting $\{F'_{th}(i)\}$. Due to the morphological imperfectness and stacking incompactness of the tiny intra-protein β -sheets, they are easier to be split than the inter-protein β -crystallites, thus to release longer protein molecules given the same external energy consumption. Besides, the limited size of intra-protein β -sheets lead to their complete destruction during stretching at forces $\{F_{tr}(i)\}$. Hereby, if the stretching force F larger than this terminating force, the intra-protein β -sheets can no longer release any length of protein molecules; instead, they are fully destroyed, and behave similarly as coiling structures in the amorphous matrix. Concerning the above statements, qualitative relation can be derived as $E_1 < E_0$, and $\langle F'_{th} \rangle < \langle F_{tr} \rangle < \langle F_{th} \rangle$, which should be well obeyed in the following modeling. Force-extension relation of spider draglines can be analogously derived as that of silkworm silk, by setting the

similar form of the lengthening term $\{\Delta L(i)\}$ for inter-protein β -crystallites in Eq.1, and a relatively more complicated form for intra-protein β -sheets shown in Eq.3.

$$\Delta L(i) = \begin{cases} 0 & F < F'_{th}(i) \\ (F - F'_{th}(i))/E_1 & F'_{th}(i) \leq F < F_{tr}(i) \\ (F_{tr}(i) - F'_{th}(i))/E_1 & F \geq F_{tr}(i) \end{cases} \quad (3)$$

The explicit force-extension expression of spider silk turns out to be:

$$F \approx \frac{k_0 L_0 \Delta x - N k_0 L_0 \cdot \Omega(F)}{N L_0 + N \Omega(F)}, \quad (4)$$

where $\Omega(F)$ denotes:

$$\begin{aligned} \Omega(F) = & \frac{p}{E_1} \left(1 - \int_0^F \rho_{tr}(f) df \right) \int_0^F (F - f) \rho'_{th}(f) df \\ & + p \cdot \int_0^F \int_0^{f_2} \left(\frac{f_2 - f_1}{E_1} \right) \rho_{tr}(f_2) \rho'_{th}(f_1) df_1 df_2 \\ & + \frac{1-p}{E_0} \int_0^F (F - f) \rho_{th}(f) df. \end{aligned} \quad (5)$$

Therein, $\rho'_{th}(\cdot)$, $\rho_{tr}(\cdot)$ and $\rho_{th}(\cdot)$ respectively characterize the distribution of the threshold forces, the critical forces terminating the intra-protein β -sheets splitting, and the threshold forces for inter-protein β -crystallites. Correspondingly, Eq.5 shows the contribution from intra-protein β -sheets splitting, their complete destruction and the splitting of inter-protein β -crystallites. The occurrence of each mechanism is also reflected in the segmentation of stress-strain profile for spider draglines. Similarly as silkworm silk, simulations have been carried out to reproduce the stretch process of spider draglines. As shown in Fig.4, the parameter ratio $E_1/(pk_0)$ and $E_0/((1-p)k_0)$ are dominant to characterize the relative steepness of stress-strain profile. Besides, the stress-strain profile is robust to the exact form of force distribution functions; instead, only their central values denoting general each mechanism in effect need to be determined. Moreover, it follows that linear elasticity k_0 and proportional constant E_0 and E_1 increases with reeling speed, indicating their dependence of initial conformation of structures. Therefore, the mechanism of each segment of the stress-strain profile of spider dragline has been clearly distinguished: the intra-protein β -sheets split at the first yielding, and their complete destruction gives rise to the lengthening of the fibrils and the stiffening in the post-yield region, thus the work-hardening phenomenon is well reproduced. On the other hand, the inter-protein β -crystallites split during or after the release of intra-protein β -sheets in the similar way of silkworm silk, resulting in their softening before breaking. The diverse high-order structures of

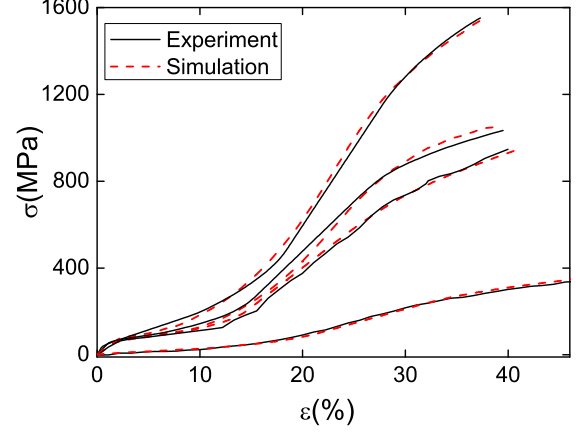


FIG. 4: The black solid lines denote the stress-strain profile for spider silk under different reeling speed[13]. Stress-strain curves of spider dragline silk (2.5, 10, 25, 100mm/sec motor-reeled at 22°C, from the bottom up) were performed using an Instron MicroTester (Model 5848; force resolution, 0.5% of indicated load; position resolution 0.02μm; strain rate is 50%/min), at 22°C and the humidity was kept at 55 – 60%. The red dashed lines are the computational results, where scaling constant $S_c = 20$, the preserved fraction of intra-molecule β -sheet $p = 0.5$, and parameters k_0 , E_0 and E_1 are varied for different reeling speeds.

protein molecules for silk fibres of different species result into their distinct mechanical complexity.

In conclusion, we have established the correlation between the nano structures and mechanical properties of silk fibres in our essay, on the basis of modeling in combination with the silk stretching experiments. We obtained for the first time the breaking mechanism of the two types of silks. The splitting of inter-protein β -crystallites gives rise to the weakening the linkage among molecules while limited capacity of intra-protein β -sheets results in the lengthening and the extra stiffening in the post-yield regime of spider draglines. Moreover, the exploration from molecular mechanical properties turns out to be a brand new probing method to investigate the subtle structural differences among biomacromolecules. This provides a new passage in understanding the origin of molecular mechanical behaviours, and will facilitate the identification of robust technologies in fabricating silks of ultrafunctionality.

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